From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK2004/000494	09.07.2004	14.07.2003

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
STATENS SERUM INSTITUT

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability: citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

   see separate sheet

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority documen was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1,12

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 1,12 are so unclear that no meaningful opinion could be formed *(specify)*:

see separate sheet

☒ the claims, or said claims Nos. 12 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, c not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2 11, 13 24, 28 38
	No:	Claims	25-27
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	2-11, 13-38
Industrial applicability (IA)	Yes:	Claims	2-11, 13-38
	No:	Claims	

2. Citations and explanations

see separate sheet

D1: RICH CHANTAL ET AL: "Identification of human enterovirulent Escherichia coli strains by multiplex PCR" JOURNAL OF CLINICAL LABORATORY ANALYSIS, vol. 15, no. 2, 2001, pages 100-103, XP008038376 ISSN: 0887-8013

D2: JP 2003 164282 A (RAKAN:KK; GIFU UNIV) 10 June 2003 (2003-06-10)

D3: EP-A-0 556 504 (SHIMADZU CORP) 25 August 1993 (1993-08-25)

D4: WO 01/94634 A (BIOPOOL INT INC) 13 December 2001 (2001-12-13)

D5: WO 02/36827 A (AUSUBEL FREDERICK M ; GEN HOSPITAL CORP (US); KUDVA INDIRA (US); CALDE) 10 May 2002 (2002-05-10)

D6: WO 03/010332 A (SCHINKINGER MANFRED ; VOLLENHOFER-SCHRUMPF SABINE (AT); FRAENZL GERT () 6 February 2003 (2003-02-06)

D7: WO 95/29261 A (UNIV HAWAII) 2 November 1995 (1995-11-02)

D8: WO 01/48237 A (HOEFT ANDREAS ; STUEBER FRANK (DE)) 5 July 2001 (2001-07-05)

D9: WO 02/053771 A (BIOTECON) 11 July 2002 (2002-07-11)

D10: WO 99/63112 A (FRASER MARK S ; HUNT WESSON INC (US); ROMICK THOMAS L (US)) 9 December 1999 (1999-12-09)

D11: WO 92/17609 A (HOLMES MICHAEL JOHN ; DYNAL AS (NO)) 15 October 1992 (1992-10-15)

D12: WO 00/61720 A (NERENBERG MICHAEL I ; EDMAN CARL F (US); METHA PRESHANT P (US); NANOGE) 19 October 2000 (2000-10-19)

D13: DE 101 23 183 A (BECTON DICKINSON CO) 22 November 2001 (2001-11-22)

D14: WO 00/29618 A (UNIVERISTY OF VIRGINIA PATENT FOUNDATION) 25 May 2000 (2000-05-25)

D15: PATON A W ET AL: "Direct detection and characterization of shiga toxigenic Escherichia coli by multiplex PCR for stx1, stx2, eae, ehxA, and saa" JOURNAL OF CLINICAL MICROBIOLOGY 2002 UNITED STATES, vol. 40, no. 1, 2002, pages 271-274, XP002304663 ISSN: 0095-1137

D16: WO 01/46477 A (CONAGRA GROCERY PRODUCTS COMPA) 28 June 2001 (2001-06-28)

Section I

Statement "floppy is identical to paper" is missing.

Section III

Independent claim 1 does not comprise technical features, but merely states a result which is to be achieved. Therefore the claim does not define the matter for which protection is sought, and does not comply with Article 6 PCT. As there are no technical features which can be compared to the prior art, claim 1 as such was not examined since no meaningful opinion could be formed with respect to novelty, inventive step and industrial applicability (Article 34 (4)(a) (ii) PCT). As all dependent claims which refer to claim 1 also refer to claim 2, the features of claim 2 are examined.

Independent claim 12 refers to an vitro diagnostic method for determining the risk of being

does not provide any such method. No embodiment is present in the application which allows the person skilled in the art to reproduce a method as claimed in claim 12, and the only technical feature of the claim, namely the detection of the ehxA gene, seems to only enable the person skilled in the art to detect infection, rather than to determine a risk of being infected. Therefore the claim seems to lack at least one essential technical feature which would be required for carrying out such a method, the claim is therefore considered unclear and not supported by the description. Hence, the claim is so unclear and so inadequadely supported that no meaningful opinion with regard to novelty, inventive step and industrial applicability could be formed (Article 34 (4)(a) (ii) PCT).

Section V

1 Novelty

1.1 Claim 25, 26

Claim 25 refers to a nucleotide sequence selected from the group consisting amongst others of the primer sequences of table 3 (SEQ ID Nos 1-25). D2 discloses a nucleic acid molecule according to SEQ ID NO 176 which is identical to a primer with SEQ ID No 2 of Table 3. Hence claims **25 and 26** are not new and do not fulfill the requirements of Article 33(2) PCT. D3 discloses nucleic sequences of 20 bp (SEQ ID No 11 and 19) which are parts of the primers with SEQ ID Nos 1 and 7 disclosed in Table 3, D4 discloses a part of 21 nucleotides of the primer with SEQ ID No 13 of Table 3 (Primer Slt1), D5 discloses a nucleic acid comprising a part of 18 nucleotides of the primer with SEQ ID No 14 of Table 3 (SEQ ID No 20). D6 discloses a nucleic acid molecule with a sequence which comprises a 16 nucleotides part of the primer with SEQ ID No 15 of Table 3 (SEQ ID No 7), D7 disclose a nucleic acid which comprises a 19 nucleotides part of the primer with SEQ ID No 17 of Table 3 (SEQ ID No 6), D8 disclose a nucleic acid which comprises a 19 nucleotides part of the primer with SEQ ID No 24 of Table 3 (Primer sknl) and D9 discloses nucleic acids which comprise the primer with SEQ ID 16 of Table 3 (Probes with SEQ ID NO 24,25 and 39). Hence, each of the documents D3-D8 destroys the novelty of claim 25 (Article 33(2) PCT).

1.2 Claim 27

D2 discloses a nucleic acid molecule which comprises 18 nucleotides of the probe sequence SEQ ID 27 of Table 7 (SEQ ID No 18). D4 discloses a nucleic acid molecule which comprises 17 nucleotides of the probe sequence SEQ ID 28 of Table 7 (SEQ ID No 178). D9 discloses a nucleic acid molecule which comprises 22 nucleotides of the probe sequence SEQ ID 30 of Table 7 (SEQ ID No 82).

D10 discloses a nucleic acid molecule which comprises 17 nucleotides of the probe sequence SEQ ID 26 of Table 7 (SEQ ID No 27). D11 discloses a nucleic acid molecule which comprises 18 nucleotides of the probe sequence SEQ ID 29 of Table 7 (primer 4). D12 discloses a nucleic acid molecule which comprises 22 nucleotides of the probe sequence SEQ ID 31 of Table 7 (SEQ ID No 45). D13 discloses a nucleic acid molecule which comprises 31 nucleotides which have at least 80% identity to the probe sequence SEQ ID 32 of Table 7 (SEQ ID No 36). D14 discloses a nucleic acid which is a 20 nucleotide part of the probe with

claim 27, which is then not new (Article 33(2) PCT).

2 Inventive step

2.1 Claim 2

D1 is considered the closest prior art for the subject matter of claim 2 and discloses a method for simultaneous detection of diarrheagenic *E. coli* groups EPEC, ETEC, VTEC (these are the strains that comprise a verotoxin or shigatoxin gene, which are in D1 referred to as EHEC), and EIEC by testing for the presence of the genes eae, vtx1 (called stx1 in D1) and vtx2 (called stx2 in D1), ipaH, sta, elt and bfpA (Tables 1 and 2). D1 implicitly detects also Shigella via the ipaH gene. In addition, the method in D1 also detects aggC and east-1 as markers for diarrheagenic *E. coli*.

Claim 2 differs from D1 in that the presence of the ehxA gene is detected along with eae, vtx1, vtx2, ipaH, sta, elt and bfpA.

No particular technical effect appears to be associated with this difference.

The problem solved by claim 2 can therefore be seen as the provision of an alternative target for detecting diarrheagenic *E.coli*.

D15 and D16 disclose the detection of ehxA in conjunction with the detection of other markers as a means to characterise diarrheagenic *E. coli* strains. D15 detects ehxA after a multiplex PCR reaction together with vtx1, vtx2, eae and saa in order to determine if a VTEC strain is more likely to be associated with severe disease (Abstract and Conclusions). In D16 a probe for the enterohemolysin encoding gene of *E. coli*, ehxA, is put on an array together with probes targeted at vtx2, eae and *E. coli* 23S rRNA (page 20; Figure 4). Therefore the use of ehxA as one of the targets for detecting diarrheagenic *E.coli* was already known from the prior art. The person skilled in the art who wanted to use an alternative target for the ones in the assay of D1, would have made an arbitrary selection amongst the targets in the prior art, and one of the possibilities would be to use ehxA as a target. The solution of claim 2 therefore cannot be considered inventive (Article 33(3) PCT).

2.2 Claim 13

A similar reasoning as above for claim 2 applies to claim 13 which is also not considered inventive (Article 33(3) PCT).

2.3 Claims 28, 29 and 30

Several documents disclose nucleic acids which are able to prime or hybridise to the genes ipaH, eae and st (e.g. in D1) or to ehxA, eae and vtx1, vtx1 (e.g. D15). To combine such nucleic acids in a kit cannot be considered inventive (Article 33(3) PCT).

A nucleic acid with a sequence as in table 7 cannot be considered inventive (Article 33(3) PCT) in view that fragments of several such nucleic acids were already disclosed (see above).